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Acquired Free Protein S Deficiency Associated With Multiple Myeloma: A Case Report

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Investigation of recurrent venous thromboembolic events in a 46-year-old man with progressive IgG kappa (total serum IgG, 74.3 mg/ml) multiple myeloma revealed profound reductions in free protein S (PS) antigen (<0.1 U/ml) and PS activity (0.33 U/ml). Total PS antigen, protein C, antithrombin III, and C4b-binding protein levels were within normal limits. The patient had no family history suggestive of a congenital PS deficiency and no history of thrombosis predating the diagnosis of his plasma cell dyscrasia. Patient IgG was isolated from serum using a protein A-sepharose affinity column and characterized. PS-dependent clotting assays (Staclot Protein S, Diagnostica Stago, Asnieres sur-Seine, France) performed on normal pooled plasma mixed with dilutions of patient IgG (0.0–33.0 mg/ml) revealed a dose-dependent neutralization of PS activity by 43%. Total and free PS antigen levels were measured using Laurell rocket electroimmunodiffusion (Assera-Plate Protein S, Diagnostica Stago), which revealed a similar dose-dependent reduction in free PS antigen but preserved normal total PS antigen. Free PS antigen was reduced by 77% to 0.23 U/ml using an IgG concentration (16.5 mg/ml) less than one-fourth of that of the patient at time of serum collection. Specific binding of the patient IgG to commercially available purified human PS was demonstrated by Western immunoblot analysis. Whereas acquired free PS deficiency has been previously reported in association with nephrotic syndrome, inflammatory bowel disease, HIV infection, and varicella infection, this is the first reported case of a hypercoagulable syndrome associated with acquired free PS deficiency and multiple myeloma. © 1996 Wiley-Liss, Inc.

Key words: protein S, multiple myeloma, thrombosis

INTRODUCTION

Protein S (PS) is a vitamin K-dependent plasma [1] and platelet [2] protein which serves as a cofactor in the protein C anticoagulant pathway [3]. PS increases the affinity of activated protein C (APC) for phospholipid surfaces [4] and thus facilitates inactivation of the nonproteolytic regulatory coagulation proteins, factor Va and factor VIIIa [5]. PS exists in dynamic equilibrium between two different forms under physiological conditions. PS can exist noncovalently associated with the multisubunit complement cascade regulatory protein C4b binding protein (C4bBP) [6]. This form normally represents 60% of total PS but lacks any anticoagulant activity. Free (unbound) PS, which represents the remaining 40% of total PS, serves as active cofactor for APC.

Congenital deficiency of PS is inherited in an autosomal-dominant pattern [7]. The majority of individuals with congenital PS deficiency have parallel reductions in total PS antigen concentration and PS functional activity (type I deficiency). Others exhibit a reduction in functional activity and a parallel reduction only in free PS antigen (type IIa deficiency). A third group of individuals has reduced functional activity without a concomitant reduction in antigen concentration (type IIb deficiency). Each type of congenital PS deficiency is associated with an increased risk of recurrent venous and arterial thromboembolism in young adults [8–10].

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A parallel reduction in PS antigen level and activity has also been described in patients with acquired PS deficiency. Acquired total PS deficiency has been described in association with disseminated intravascular coagulation [11], warfarin therapy, oral contraceptive pill use [12], pregnancy [13], and L-asparaginase chemotherapy [14]. Acquired PS deficiency has also been described in a child with varicella infection, life-threatening thrombosis, and the transient production of noninhibitory, monoclonal antibodies capable of binding to purified PS in vitro [15]. There are limited data to support an association between acquired PS deficiency and a thrombotic diathesis.

A selective acquired deficiency of free PS has been reported in conjunction with human immunodeficiency virus (HIV) infection [16], nephrotic syndrome [17], inflammatory bowel disease [18], and other systemic inflammatory disorders associated with an increase in acute phase reactant C4bBP. We report on a case of acquired free PS deficiency in a patient with progressive IgG kappa multiple myeloma, recurrent venous thrombotic events, production of a monoclonal IgG capable of neutralizing PS cofactor activity in a dose-dependent manner, and a normal level of C4bBP.

CASE REPORT

A 46-year-old Caucasian man with a 6-year history of IgG kappa multiple myeloma was evaluated for a hypercoagulable condition because of recurrent venous thrombosis. The patient was diagnosed with multiple myeloma in 1985 following the detection of an increased total serum protein-to-albumin ratio. A serum protein electrophoresis revealed an M-protein, immunoelectrophoresis revealed a monoclonal IgG kappa antibody, and a bone marrow biopsy revealed 20% plasma cells in sheets. Following a hospital discharge in September 1991, he developed profound dyspnea. A ventilation/perfusion lung scan revealed multiple mismatched segmental and subsegmental defects which were interpreted as high-probability for pulmonary embolism. Lower extremity noninvasive vascular imaging studies of both legs were unrevealing. He was treated with intravenous heparin followed by coumadin therapy to maintain his prothrombin time between 1.5–2.0 times control. Four weeks into this course of oral anticoagulation therapy, he developed right leg swelling secondary to a new distal superficial femoral and popliteal vein thrombosis, diagnosed by Doppler and compression ultrasound. Four months after completing a 6-month course of oral anticoagulation, he was hospitalized because of chemotherapy-associated neutropenic fevers. During this hospitalization, a new left lower extremity deep venous thrombosis was documented and treated with anticoagulants. A hypercoagulable workup performed at this time was most notable for free PS deficiency. He had no family history suggestive of a congenital thrombophilia, and no history of documented thrombosis prior to the diagnosis of plasma cell dyscrasia. Family members were not available for testing. His serum IgG progressively increased (33.0 to 74.3 mg/ml) between 1991–1993.

MATERIALS AND METHODS

IgG Purification

Patient IgG was purified from thawed serum on an affinity column containing protein A sepharose (Sigma, St. Louis, MO) with a recovery rate of 28%. Bound IgG was eluted with 0.1 M glycine, pH 3.0, dialyzed against 0.05 M Tris-HCl, pH 7.4, and 0.15 M NaCl, and concentrated in a Centriprep 30 (Amicon, Danvers, MA).

Protein S Activity Assays

PS activity was determined by a clotting assay based upon the cofactor activity of PS which enhances the anticoagulant action of protein C (Staclot Protein S, Diagnostica Stago, Asnieres-sur-Seine, France) [19]. This enhancement is reflected by the prolongation of clotting time in a system enriched with factor Va, a physiological substrate for APC. Assays were performed using pooled normal plasma (Precision Biologicals, Halifax, Nova Scotia, Canada) incubated for 1 hr at 37°C with serial dilutions of either concentrated patient IgG (final concentration, 0.0–33.0 mg/ml) or pooled normal human IgG (Sigma).

Protein S Antigen Assays

Total and free PS antigen levels were measured using Laurell rocket electroimmunodiffusion (Assera-Plate Protein S, Diagnostica Stago) [20]. Assays were performed on pooled normal plasma incubated at 37°C with serial dilutions of concentrated, purified patient IgG (0.0–16.5 mg/ml) or pooled human IgG (16.5 mg/ml). PS contained in the test sample was made to migrate under the influence of an electric field in an agarose gel in which specific antibodies to PS had been incorporated. Free PS was determined by the same general method after the addition of 25% polyethylene glycol, incubation for 30 min at 4°C, and centrifugation for 10 min at 3,000*g* to precipitate C4b-BP-bound PS.

Immunoblotting

Purified human PS (1.0 μg of unreduced PS per lane) (Haematologic Technologies, Inc., Essex Junction, VT) was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [21] and transferred to a 0.45-μM PVDF transfer membrane (Immobilon-P, Millipore, Bedford, MA) for electroblotting at a fixed voltage (100 V) for 1 hr at 4°C. After blocking with 0.05 M Tris-HCl, pH 7.4, 0.15 M NaCl, and 5% dry milk for 30 min at room temperature, the membrane was cut into

strips and exposed for 2 hr at 37°C to either the patient's purified IgG fraction (adjusted to a final total concentration of 12.5 mg of IgG/ml), pooled human IgG (12.5 mg/ml) (Sigma), or a purified mouse monoclonal IgG_1 which recognizes human PS and PS-C4bBp complexes (Haematologic Technologies, Inc.). After washing with blocking agent, each membrane strip was incubated for 2 hr with either alkaline phosphatase-conjugated goat antibody specific for human IgG Fc (Sigma) at a dilution of 1:5,000 or alkaline phosphatase-conjugated goat antibody to mouse total IgG (Sigma) at a dilution of 1:5,000. Each membrane strip was developed by the addition of the alkaline phosphatase substrate 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt and p-nitro blue tetrazolean chloride (BCIP/NBT Color Development Solution, Bio Rad, Hercules, CA) for 10 min.

Other Procedures

Antithrombin III activity (Spectrolyse Antithrombin III, Biopool, Burlington, Ontario, Canada), antithrombin III antigen (Liatest AT III, Diagnostica Stago), C4b-BP (Liatest C4b-BP, Diagnostica Stago), protein C antigen (Protein C Antigen Rocket EID Method, Helena Laboratories, Beaumont, TX), and anti-phospholipid antibodies (IgM and IgG) (Asserachrom APA, Diagnostica Stago) were measured in the patient's plasma. Purity and light-chain specificity of the purified patient IgG were determined by serum protein electrophoresis and immunoelectrophoresis (Paragon IFE Gel, Beckman, Brea, CA).

RESULTS

In August 1992, while the patient was being treated with warfarin anticoagulation for his second documented deep vein thrombosis, an evaluation revealed antithrombin III (antigen, 1.18 U/ml; anticoagulant activity, 1.02 U/ml), protein C antigen (0.73 U/ml), and plasminogen antigen (1.03 U/ml) which were all within normal reference range. An anti-phospholipid antibody screen was negative. The patient's total PS antigen level was 0.80 U/ml (normal range, 0.67–1.67 U/ml); whereas, his free PS antigen was markedly reduced at less than 0.10 U/ml (normal range 0.53–1.21 U/ml). Repeat evaluation in January 1993, in the absence of pharmacologic anticoagulation, again revealed normal concentrations of antithrombin III (antigen, 0.84 U/ml; anticoagulant activity, 1.04 U/ml), protein C antigen (0.68 U/ml), and plasminogen antigen (1.26 U/ml). A repeat anti-phospholipid antibody screen was negative. The total PS antigen level was normal at 0.70 U/ml. Both free PS antigen and PS anticoagulant activity were markedly decreased at <0.10 U/ml and 0.33 U/ml, respectively. The level of C4bBP was normal.

One ml of patient serum (IgG concentration, 51.8 mg/ml) was purified over a protein A-sepharose affinity column, resulting in the elution of 14.45 mg of IgG kappa antibody as determined by immunoelectrophoresis (data not shown). The IgG kappa was dialyzed into a suitable buffer at physiologic pH, and concentrated to a final volume of 0.4 ml with a concentration of 33.0 mg/ml. This final concentration was approximately equal to the patient serum IgG concentration at the time of his first documented thrombotic episode and half the patient IgG level at the time of his hypercoagulation evaluations.

The patient's purified IgG fraction inhibited the APC cofactor activity of PS in a dose-dependent manner. The PS activity of pooled normal plasma was reduced from 1.00 U/ml to 0.57 U/ml when the test plasma was adjusted to 33.0 mg/ml of patient IgG (Fig. 1). A similar dose-dependent reduction in PS anticoagulant activity was found when pooled normal plasma was mixed with complete patient serum (data not shown). Increasing concentrations (up to 33.0 mg/ml) of the IgG fraction of pooled normal plasma had no inhibitory effect.

The addition of patient purified IgG (up to a final concentration of 16.5 mg/ml) to pooled normal plasma did not result in any reduction in total PS antigen level. On the other hand, the free PS antigen level was decreased in a dose-dependent manner. Free PS antigen was reduced from 1.00 U/ml to 0.23 U/ml by a concentration of patient IgG (16.5 mg/ml) less than one fourth the patient's serum IgG concentration during the final year of his illness (Fig. 1). The addition of IgG from pooled normal plasma to a final concentration of 16.5 mg/ml did not reduce the total of free PS antigen level below the lower limit of normal.

We objectively demonstrated the binding of patient IgG antibody to purified PS by transferring PS to PVDF membranes and incubating the membranes with the patient's serum IgG fraction (Fig. 2). Patient IgG bound to blotted PS was visibly detectable following the addition of alkaline phosphatase conjugated goat antihuman IgG Fc antibody and a suitable developing reagent. Bound patient IgG was detected at sites which correspond to a PS standard and C4bBP. Pooled normal human IgG only bound to sites consistent with C4bBP suggestive of a non-specific interaction.

DISCUSSION

We describe a novel case of acquired PS deficiency associated with a circulating, inhibitory antibody targeted against free PS in a patient with progressive IgG kappa multiple myeloma and recurrent venous thrombosis. The patient's quantitative IgG level at the time of his initial thrombotic event correlated with the minimum IgG concentration needed to neutralize PS cofactor activity below normal in vitro. It is likely that his initial thrombotic event occurred at a time of moderate free PS deficiency which progressed to a more severe degree as his multiple myeloma evolved and his paraprotein level increased. Total PS antigen, protein C antigen, and other vitamin

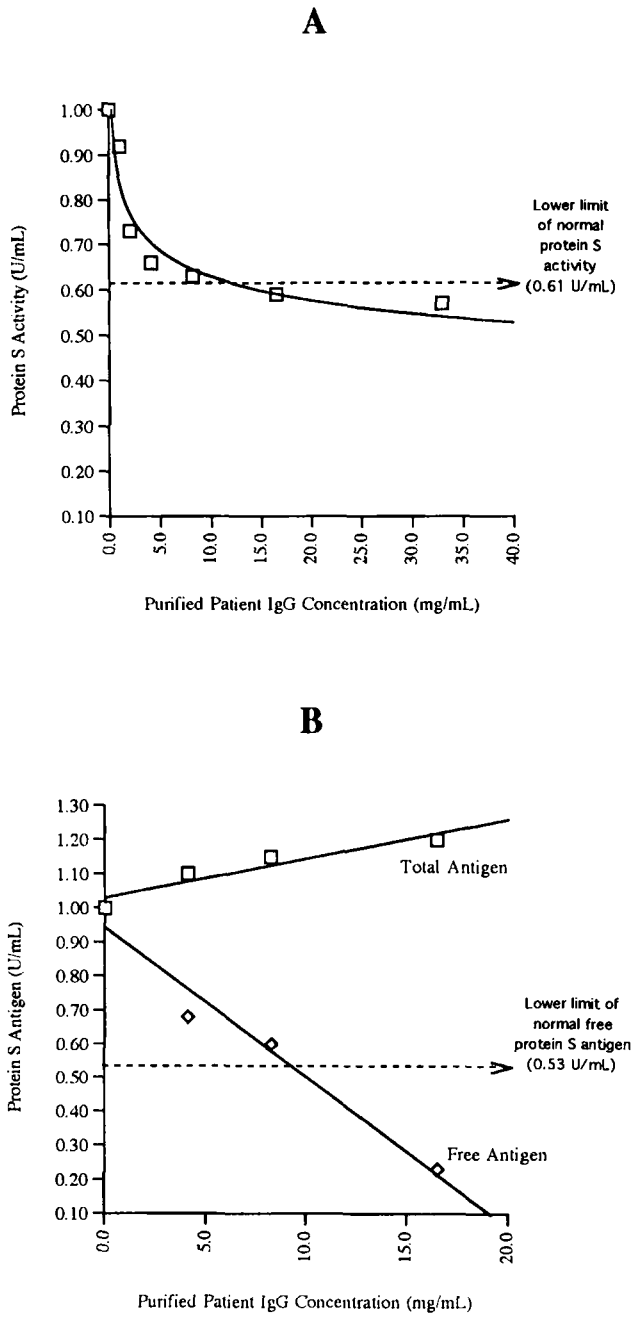

Fig. 1. Plasma PS activity neutralization following incubation with purified patient IgG in final concentrations ranging from 0.0 to 33.0 mg/ml (panel A) and plasma PS total and free antigen neutralization following incubation with purified patient IgG in final concentrations ranging from 0.0 to 16.5 mg/ml (panel B).

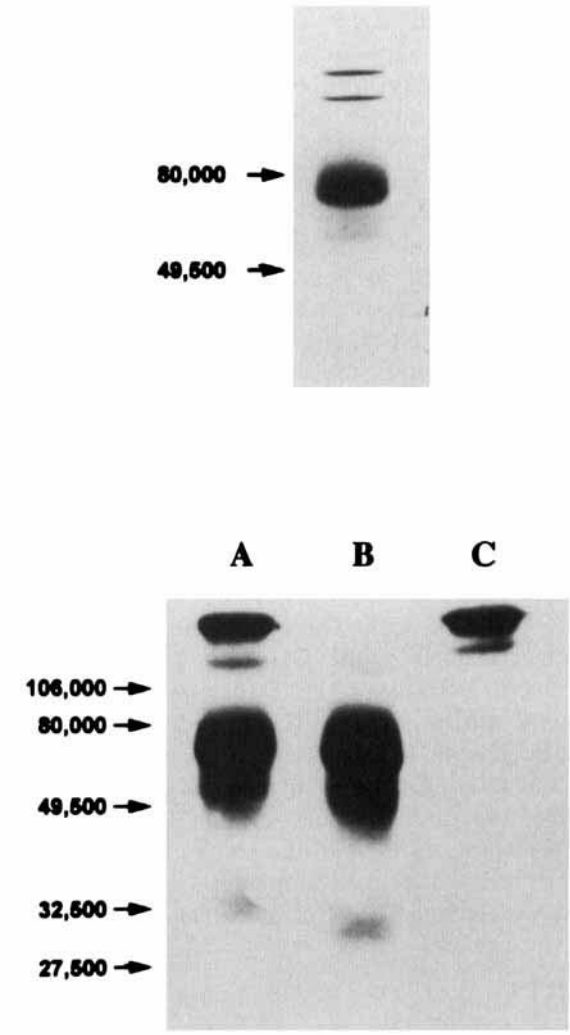

Fig. 2. Detection of IgG antibody to protein S by immunoblotting. SDS-PAGE of purified human PS (1.0 μg) stained with coomassie brilliant blue (upper panel). Immunoblots of PVDF membrane bound purified PS incubated with patient purified IgG at a final concentration of 12.5 mg/ml (lane A), purified mouse monoclonal IgG_1 which recognizes human PS and PS · C4bBp complexes at a final concentration of 12.5 mg/ml (lane B), and pooled human IgG at a concentration of 12.5 mg/ml (lane C) (lower panel).

K-dependent coagulation factors were not affected. The C4bBP level was normal; thus, a marked shift of PS from the free (active) pool to the bound (inactive) pool induced by an increase in C4bBP seems unlikely.

Thrombosis in association with multiple myeloma and related disorders has been described, but is traditionally attributed to patient immobility, low-grade disseminated intravascular coagulation (DIC), anti-phospholipid antibodies, or hyperviscosity [22]. Hypercoagulability in conjunction with multiple myeloma and a circulating inhibitor of a natural anticoagulant, protein C, has been described in one patient to date [23]. This case report is the first description of PS deficiency in association with a paraprotein-producing B cell disorder.

Monoclonal immunoglobulins may affect coagulation by acting as true antibodies which bind to specific epitopes on coagulation proteins by way of their antigen binding sites, or they may bind nonspecifically. These

interactions may result in inhibition of hemostatic or anticoagulant function, or may result in increased clearance of the complex, causing an apparent deficiency state. In the latter case, the addition of normal plasma to patient plasma would be expected to correct the coagulation defect in vitro. In our case, the addition of normal plasma did not correct the anticoagulant defect. The observed reduction in free PS antigen in the absence of a similar reduction in total PS antigen may indicate that the antibody was not only inhibitory in nature but also capable of enhancing free PS clearance in vivo. This selective reduction could also reflect immune complex formation and the subsequent removal of free PS in vitro during polyethylene glycol precipitation prior to immunoelectrophoresis.

We have demonstrated a specific inhibitory interaction between a paraprotein and a natural anticoagulant protein which precipitated thrombosis. Our demonstration of this interaction, in addition to the report by Gruber et al. [23], suggests that an association between multiple myeloma and thromboembolic disease may not be coincidental. We recommend that patients with plasma cell dyscrasia who present with thrombosis receive a careful clinical and laboratory evaluation in order to facilitate the prompt institution of appropriate therapy.

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